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THE TEMPERATURE FACTOR IN FERTILIZATION AND GROWTH OF THE BARLEY OVULE[1]

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INTRODUCTION

Temperature has long been recognized as a factor that profoundly influences growth. Minimum, optimum, and maximum temperatures have been determined in many plants for different phases of growth and have been found to differ for different species, for different organs in the same plant, and for different ages of the organ. Moreover, a definite variation occurs with the length of exposure.

As early as 1861, Hofmeister (*5*) [2] had observed that in *Crocus vernus*, under favorable conditions of warm moist air and bright sunshine during the daylight hours, the pollen tube reached the micropyle in 24 hours, whereas in cooler, drier air its passage required 48 to 72 hours. In *Trifolium pratense*, Martin (*7*) found that, during the high temperatures of July, division of the egg cell occurred 18 hours after pollination, whereas, in the much cooler October, the time required was 35 to 50 hours. Shibata (*10*) stated that he was able to lengthen the time between pollination and fertilization at will in *Monotropa uniflora* by lowering the temperature, and that almost complete suppression of fertilization occurred at 8° to 10°. He found that fertilization and early development proceeded as well at 28° as at "room temperature," with endosperm division continuing up to 30°. However, at 31° to 32° these processes were entirely suppressed.

Smith and Cochran (*11*) found that tomato pollen germinated best with pollen tube growth at its maximum for 6 to 12 hours at 85° F. (29.4° C.), after which the sample kept at 70° outstripped it. In studying the rate of pollen tube growth for the first 12 hours in *Datura*, Buchholz and Blakeslee (*3*) found that growth was 4½ times as fast at the optimum (33.3° C.) as at 11.1° C. At 37°, the highest temperature used, a slight falling off in the rate was observed.

In the cereals, the period of heading is critical. Unfavorable conditions at this time, such as hot winds, drought, or cold rainy weather, may be followed by lowered yields resulting from sterile florets or blasted spikes. The incidence of floret sterility in the Great Plains during the years 1910–12 led Salmon [3] to conclude that high temperatures alone at or slightly before heading could result in sterile florets in normal-appearing spikes. Later, he (*9*) found that temperatures of 30° to 40° C. at flowering gave high sterility in wheat.

[1] Received for publication May 18, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 402.

[3] Data on Report of Cereal Investigations at the Bellefourche Experiment Farm, Newell, S. Dak., 1912, on file with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

In rice, which is a warmer weather cereal, Kondō and Okamura (*6*) stated that 30° is optimum for the flowers.

The present study was limited to a measurement of temperature effects on a few phases of growth in a single variety of barley during the first 6 days following pollination.

MATERIAL AND METHODS

In February 1940, spikes of Manchuria barley (C. I.[4] 2330), a variety of *Hordeum vulgare* var. *pallidum* Ser. growing in the greenhouse at the Arlington Experiment Farm, Arlington, Va., were prepared, emasculated, and bagged. They were allowed to remain on the plant 4 days at an average temperature of about 14° C.; at the end of this time the flowers were well opened, with stigmas spread wide. Their culms were cut off just below the third node from the spike. These culms, each having two foliage leaves and the basal half of the boot leaf, were distributed among five Erlenmeyer flasks with their cut ends in distilled water. One flask was placed in each of five chambers carried at 10°, 15°, 20°, 25°, and 30°, respectively, and allowed to approach temperature equilibrium for 50 to 60 minutes. The spikes were then inserted into a paper cone and pollinated by dusting with heads of the same variety of barley bearing dehiscing anthers; they were then allowed to continue their growth, as earlier investigations (*8*) had shown that, in the barley embryo, the rate of growth for the first 11 days after pollination was practically the same in culms excised and placed in distilled water as in those left on the plant, when the two lots were kept side by side on the greenhouse bench. Pollinations were spaced 2 minutes apart so as to allow time to take the sample and move to the next chamber. Sampling was begun 20 minutes after pollination and continued at 10-minute intervals up to 90 minutes, and thereafter at intervals of 4, 5, and 6 hours and 1, 2, 3, 4, 5, and 6 days after pollination. All samples, consisting for the most part of five ovules, were in the killing fluid within about 1 minute of the interval indicated except on the fifth day, when the sampling was about 6 minutes late.

Good control of temperature was obtained in all chambers except that for 30° C., a small tank open at the top, which varied between 28° and 31° at sampling time. The chambers were dark except where light bulbs for heating were placed behind baffle boards. Relative humidity was not recorded. The ovules seemed to develop normally in all the chambers. In general, basal and tip ovules were rejected but every spike in a chamber was represented in a single sample. Formalin acetic alcohol was used as a killing and fixing fluid because of its relatively low surface tension and rapid penetration. Fixation was fair, but not up to the standard desired for cytological work, as in many samples the contents of the embryo sac were in abnormal positions.

The ovules were embedded in paraffin, cut sagittally 15μ thick, and stained, usually with Heidenhain's iron-alum haematoxylin. The process was greatly shortened in many of the older samples by mordanting briefly in iron alum and staining with 1/80 percent haematoxylin until the cell walls were blue.

[4] C. I. refers to accession number of Division of Cereal Crops and Diseases.

A study of the 1940 results showed that additional data were desirable, so the experiment was repeated in 1941 for the temperatures 10° and 30° C., and samples run at 5°, 35°, 40°, and 45° were added. Extra samplings were made at ages shown or suspected to be critical. The 5°, 10°, and 30° chambers, located in constant-temperature rooms in the basement of the greenhouse, held their temperatures satisfactorily, but those at 35°, 40°, and 45°, which were small tanks located in the greenhouse under glass and heated by light bulbs, varied more widely, the 35° and 40° tanks running 1° to 2° high. Excessively dry atmospheric conditions were guarded against by placing open pans of water in the 30°, 35°, 40°, and 45° chambers.

Much better fixation was obtained in 1941 by pre-fixing in acetic alcohol and completing the process in Craf. Both years' work will be considered together. Observations are based on about 1,000 ovules sectioned and stained.

EXPERIMENTAL RESULTS

Flower closing after pollination was faster with increased temperature. In the first year's experiments at 25° and 30° C., closure was complete in 1 day; in those at 15° and 20° the flowers were closed on the second day; and in those at 10°, on the fourth. After 6 days in the dark, the green color of the foliage had faded perceptibly at the higher temperatures. Alcoholic extracts of leaf tissue from homologous selected culms at the five temperatures used in 1940 showed the 10° samples to be the greenest. Using a Duboscq-type colorimeter and the 10° value as 100, the 15° sample had faded to 89.9, the 20° sample to 82.2, the 25° sample to 74.3, and the 30° sample to 72.9.

GROWTH OF POLLEN TUBE

Figure 1 shows the shortest time after pollination at which male nuclei were seen in the egg sac. At 5° the pollen tube grew very slowly, taking 140 minutes to reach the embryo sac, and the male nuclei had not made contact with the female nuclei at the end of 180 minutes. The rate of growth increased rapidly with temperature up to a maximum at 30° and 35°, when but 20 minutes were required to reach the embryo sac. At 25° the earliest occurrence of male nuclei in the egg sac was seen in the 30-minute sample, but they were then in contact with the egg and polar nuclei. At 40° no male nuclei were visible in any of the 10 ovules sampled at 20 minutes, but out of 6 ovules sampled at 30 minutes, 1 showed their presence. At this temperature, the egg and polar nuclei appeared abnormal in the first sample taken (10 minutes after pollination, or about 70 minutes' exposure to 40°). Later samples showed progressive degeneration. The effect of heat on the 45° samples was still more marked, and no evidence was found of male nuclei reaching the embryo sac at that temperature.

INCREASE IN NUMBER OF NUCLEI IN EMBRYO AND ENDOSPERM

The fertilization process, as observed in the barley ovules, is in general as follows. The two male nuclei reach their objectives at very nearly the same time. The group of three nuclei (1 ♂ and 2 ♀ polar nuclei) that is soon to produce the fusion nucleus then

migrates rather rapidly along strands of protoplasm until it takes a position at the proximal end of the mass of antipodal cells near the center of the embryo sac. Fusion and division into two daughter endosperm nuclei without cell walls then occur. These migrate along

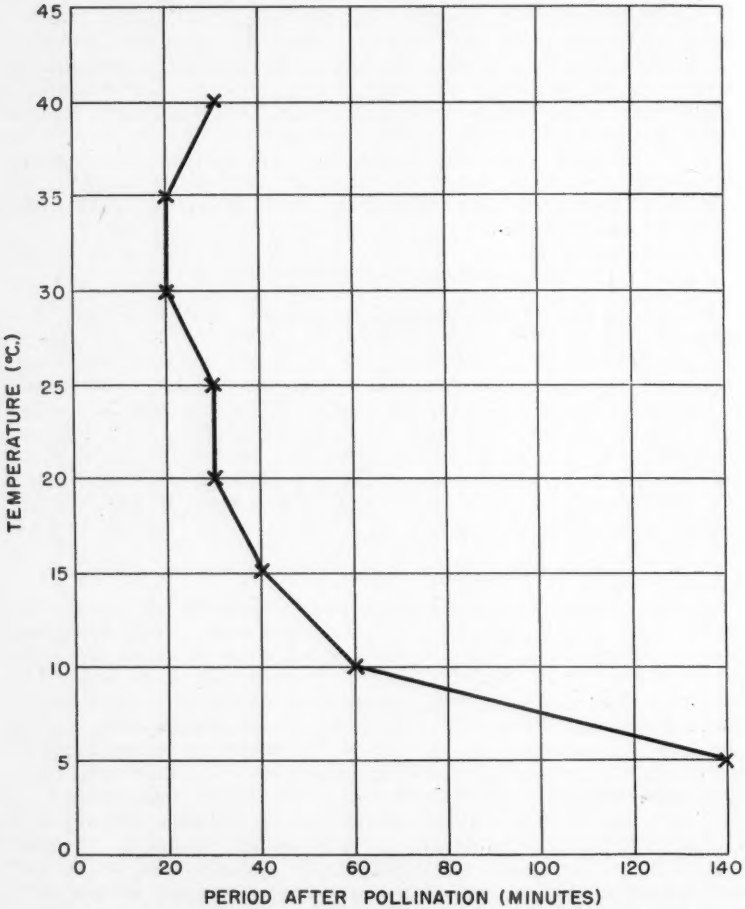

FIGURE 1.—Time from pollination required for male nuclei to enter the embryo sac of Manchuria barley when grown at constant temperatures.

the protoplasm strand and divide again. As ovule growth proceeds these protoplasmic strands gradually become a sheet of protoplasm lining the embryo sac cavity and affording free living room for a number of generations of dividing nuclei. For several generations the endosperm nuclei divide almost simultaneously and increase in number exponentially or according to the compound-interest formula

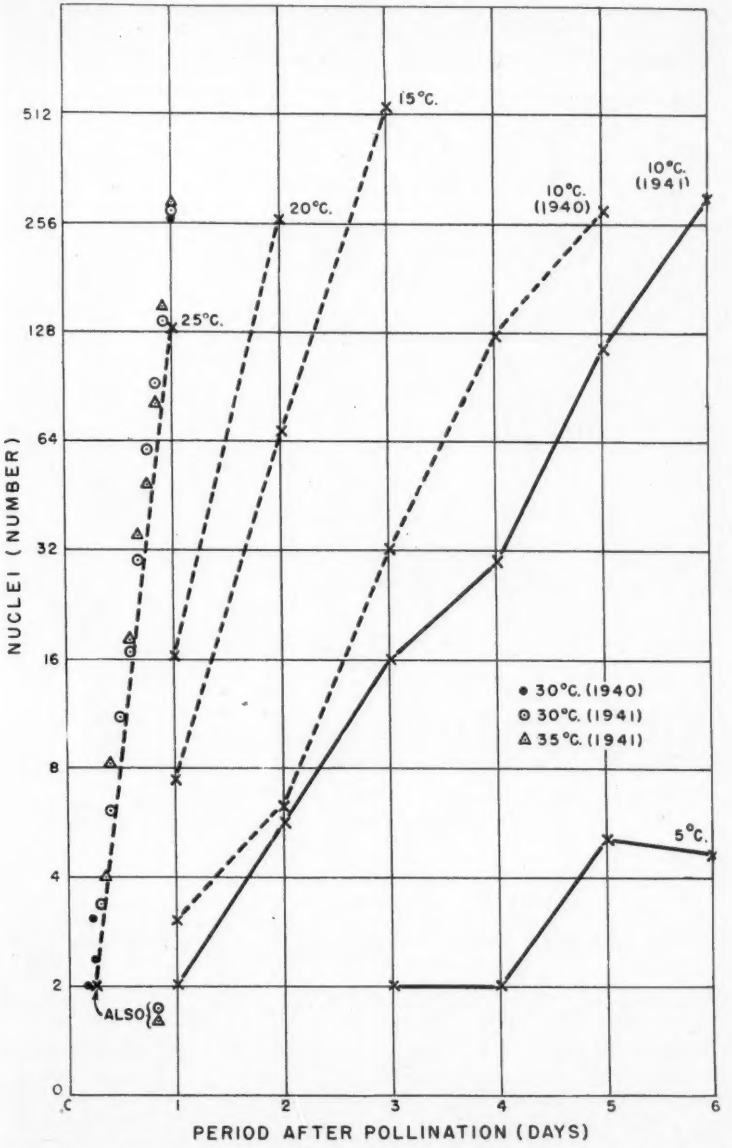

FIGURE 2.—Increase in number of endosperm nuclei in ovules of Manchuria barley growing at constant temperatures.

at 100 percent. The fertilized egg exhibits polarity from the first, division being much more frequent in the distal half. Unlike the developing endosperm, the embryo cells have cell walls. The rate of division in the young embryo is also according to the compound-

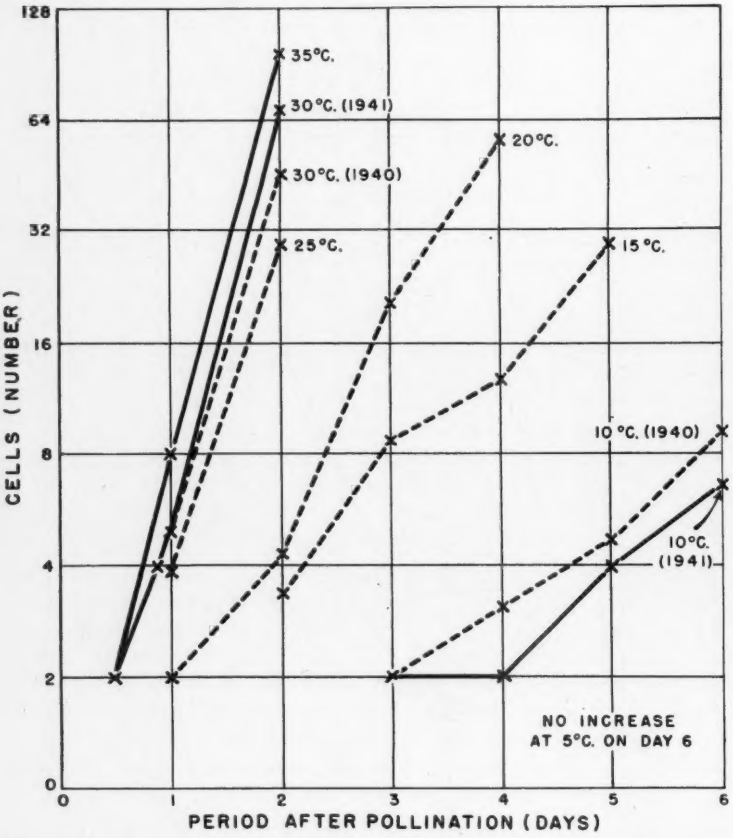

FIGURE 3.—Increase in number of embryo cells in ovules of Manchuria barley growing at constant temperatures.

interest formula, but because of its polarity, not at the 100-percent rate.

Endosperm and embryo nuclei at successive ages were counted under the microscope, but, since mitosis in the nucleus is a rapid process between two relatively long rest periods, the number of nuclei at any time may be double or but half the number that would have appeared had the sample been taken an hour or so later or earlier. The fact that nuclei were often divided by the microtome knife made exactness of counting almost impossible in the later stages, but it is believed that the numbers shown are consistent and sufficiently accurate.

The counts have been plotted with successive powers of 2 used as ordinates, which would be the number of generations where increase in number of nuclei equals compound interest at 100 percent. In the endosperm, as shown in figure 2, nucleus increase was very slow at 5° C., reaching a maximum of less than 8 nuclei, or 3 generations, in 6 days. At 10°, 9 generations occurred in 6 days in 1941, and in 5 days in 1940. At 15°, there were 10 generations in 3 days; at 20°, 8 generations in 2 days; and at 25°, 7 generations in 1 day. The increases at 30° C. for both years, and for 35° are practically

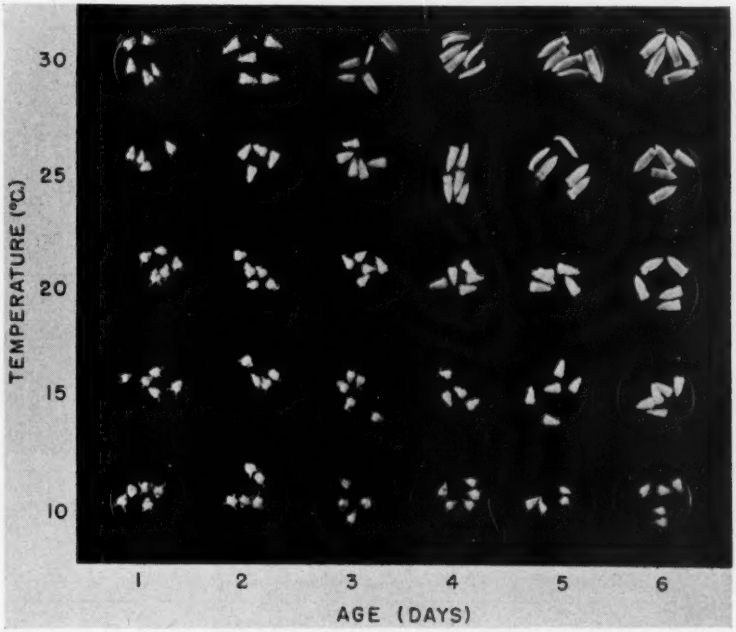

FIGURE 4.—Ovules of Manchuria barley grown at constant temperatures and harvested at daily intervals.

identical, or 8 generations at 1 day after pollination. No evidence of endosperm division was found in the 40° and 45° samples.

The curves of embryo cell increase (fig. 3) show a much slower growth than was shown in the case of the endosperm. There was no division at 5° C. in 6 days, but rate of growth increased rapidly with temperature until at 35° there were 7 generations in 2 days. At 40°, 2 young embyros were found, a 2-celled one at 1 day, which was dying, and 1 of about 46 cells, after 4 days' growth, which appeared to be dead. No embryos were found at 45°.

By way of comparison, germination tests were made of the Manchuria seed in Petri dishes in constant-temperature chambers. The sample kept at 15° C. germinated perfectly; 42 percent of the seed at 35° germinated weakly; and none germinated at 40°.

INCREASE IN LENGTH OF CARYOPSIS AND EMBRYO

The effect of temperature on kernel growth was very marked for 6 days after pollination. A photograph made of the 1940 samples is

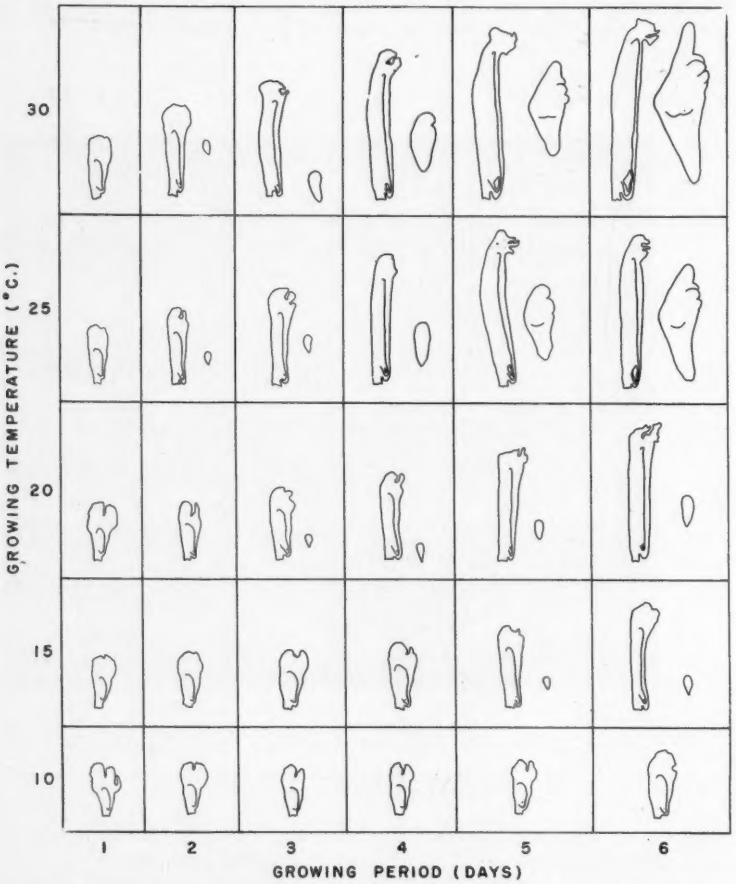

FIGURE 5.—Sagittal sections of typical ovules of Manchuria barley and their embryos grown at constant temperatures and harvested at daily intervals. Ovules, ×3.9; embryos, ×29.

shown in figure 4. The average length of the ovules grown at 30° C. for 6 days was approximately 7.02 mm.

The ovules shown there were sectioned sagittally and stained, and the median section of each was drawn with the aid of the camera lucida. Where the number of cells in the embryo exceeded 20, it too was drawn at a greater magnification. The lengths of these were determined and the ovules with their embryos most nearly approaching the average for each sample were taken as typical, and from them a chart was

constructed, as shown in figure 5, which should be useful in obtaining samples of definite stages.

After the lengths of the caryopsis and embryo were obtained from the 1941 data, the averages of the samples were plotted for all the temperatures used. The curve for caryopsis length (fig. 6) shows

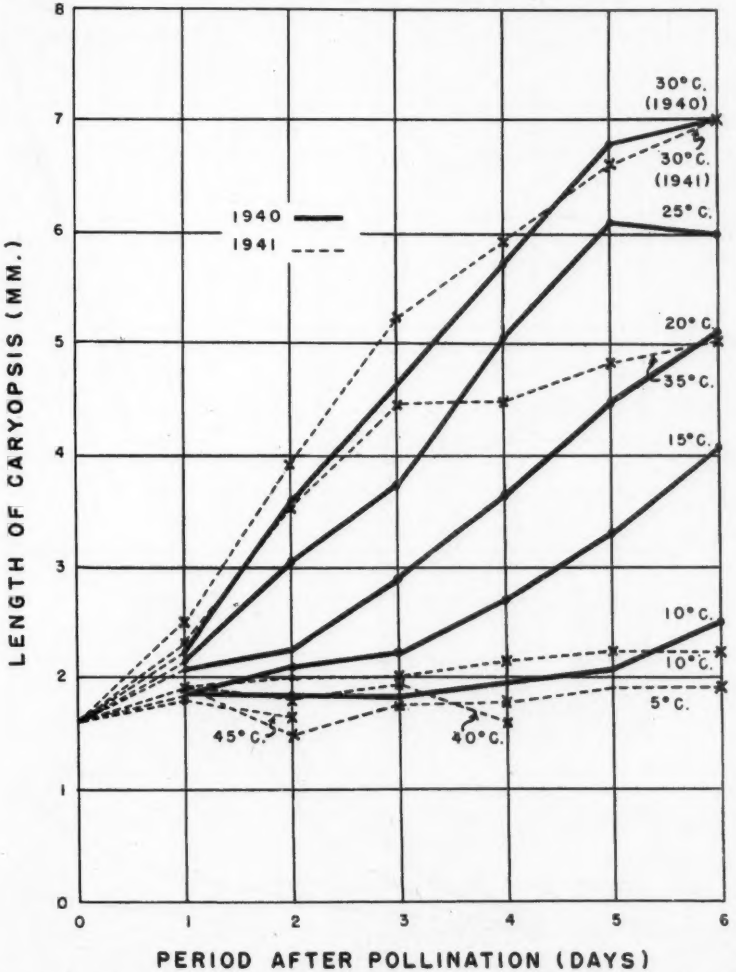

FIGURE 6.—Increase in length of caryopsis in Manchuria barley growing at constant temperatures.

very slight growth during 6 days at 5° and 10° C., but rapidly increasing growth with successively higher temperatures to a maximum at 30°. Growth at 35° was about equal to that at 30° for 3 days, but dropped rapidly thereafter. Little or no growth occurred at 40° and 45°.

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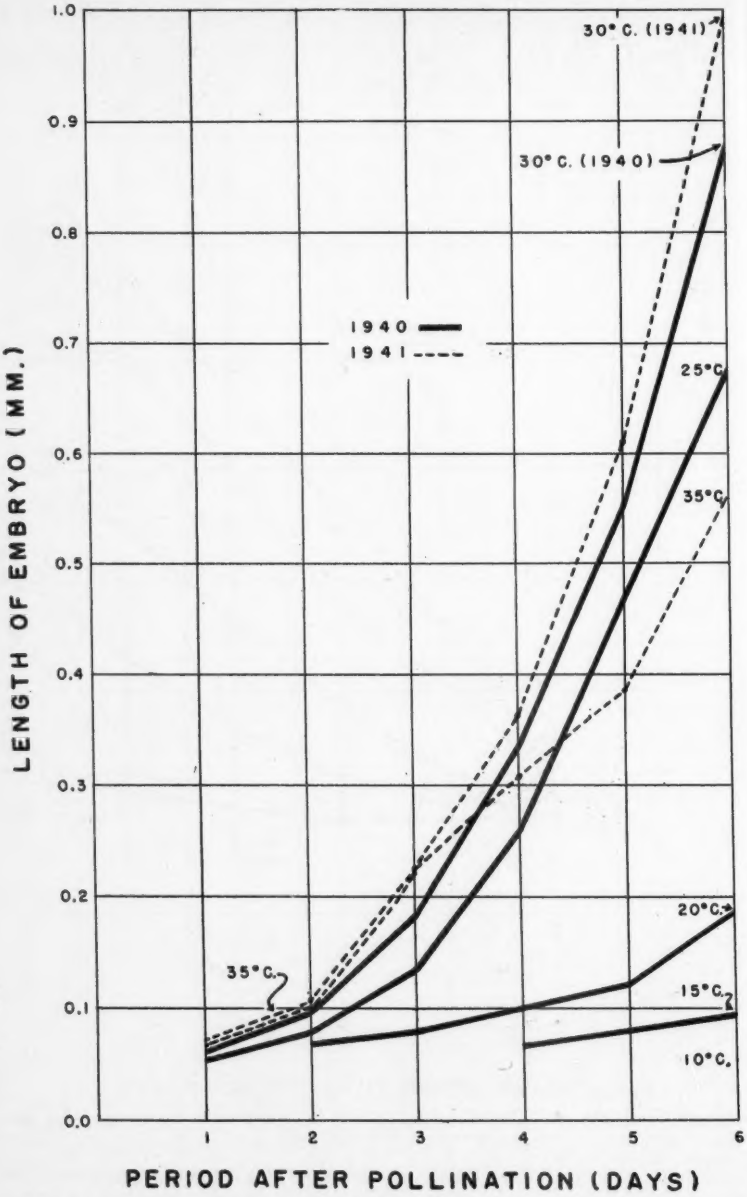

FIGURE 7.—Increase in length of embryo in Manchuria barley growing at constant temperatures.

In the embryo, the first division had not taken place after 6 days at 5° C. (fig. 7). At 10° and 15°, growth was very slight.
As in the caryopsis, the maximum length was attained at 30° and growth at 35° fell off after 3 days. No curves are shown for embryo

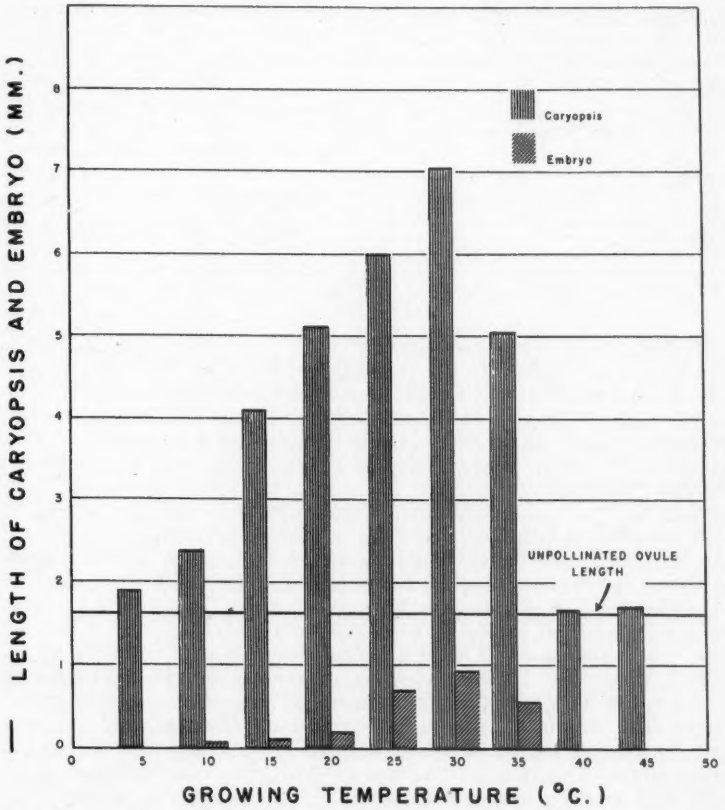

FIG. 8.—Length attained in 6 days at different growing temperatures by the caryopsis and the embryo of Manchuria barley.

growth at the two highest temperatures since fertilization was exceptional at 40° and absent at 45°.

Figure 8 compares the finally attained lengths of caryopsis and embryo at the various temperatures.

Van't Hoff's rule that velocity of a chemical reaction in homogeneous systems is doubled or trebled with an increase in temperature of 10°, C. has been found to apply to many vital processes at temperatures below the thermal death point. In table 1 are given the temperature coefficients calculated for the 10° intervals available, on the basis of increase with temperature in 6 days in number of generations of endosperm and of embryo nuclei, and in growth in length of ovule and embryo.

It is seen that the endosperm divided 5.5 times as fast at 15° C. as at 5°, but both endosperm and embryo divisions showed good agreement with the rule in the next three intervals, while at 25° to 35° the rate fell off greatly. On the other hand, growth in length of ovule and embryo showed only one close approximation; i. e., for the interval 15° to 25° in the ovule.

TABLE 1.—*Van't Hoff's temperature coefficient (Q10) for growth in Manchuria barley*

Temperature interval (°C.)	Number of Generations		Length	
	Endosperm	Embryo	Ovule	Embryo
5–15	5.5		8.3	
10–20	2.4	2.5	4.7	3.6
15–25	2.1	2.5	1.8	7.1
20–30	2.1	2.2	1.5	5.0
25–35	1.3	1.4	.8	.6

DISCUSSION

The minimum temperature for growth in the pollen tube and ovule of barley was apparently not reached at 5° C. At this temperature, fertilization progressed normally, but with no divisions in the embryo at the end of 6 days. In the endosperm, two to three divisions had taken place and the ovule had made a slight growth in length. With increasingly higher temperatures, the growth rate was markedly accelerated until at 30° and 35° the pollen tubes had completed their growth and extruded the male nuclei into the egg sac within 20 minutes after pollination. At both of these temperatures, growth of the ovule and embryo was at its maximum for the first 3 days. Since at this point the curves of the 35° samples suffered inflection, we may conclude that 30° is about the optimum temperature for these organs for a 6-day period, while 35° is close to the maximum. A similar high rate suffering early inflection was observed by Famin (*4*) in the growth of roots and shoots of *Pisum* where an initial maximum rate found at 29° later fell off and was exceeded by that of seedlings growing at 22°. Higher temperatures gave rates following much the same pattern until at 35° to 36° there was little or no growth. In the experiments of Kondō and Okamura (*6*) with rice, tillering was best at 34.5° and length growth at 30° to 32°. The rate fell off progressively at 36°, 37.5°, and 39°, and temperatures of 43.5° and 48° were lethal.

In the studies of barley reported herein, the thermal death point was reached at 40° C. Although the male nuclei were seen in one embyro sac killed 30 minutes after pollination and two very young embryos were present in later samples, no endosperm nuclei were found and no ovule appeared to be living after an estimated maximum development of 20 hours. After the spikes were given an hour's conditioning exposure before pollination, six of the seven ovules in the 40° sample taken 10 minutes after pollination showed abnormalities in the egg and polar nuclei. Progressive degeneration was evident in the egg sac of later samples. Although the flowers had been subjected to the mutilation ordinarily practiced in emasculation, and the unnaturally open interiors were exposed to the experimental temperature

for a longer time than the pollen, the evidence suggests that sterility at high temperatures may be due as much to ovule injury as to pollen killing.

From the counts of endosperm and embryo nuclei at each temperature during the first 6 days after pollination, it was found that there are approximately three divisions of the endosperm to one of the embryo. This striking difference in growth rate can hardly be explained on the basis of nutritional opportunity, since even under initially almost identical conditions the first division of the fertilized egg does not occur until there have been about three divisions in the endosperm, and during the whole time that the nuclei are countable the same ratio of division persists. Theoretically, all eight of the nuclei in the embryo sac are identical as are the two sperm nuclei; hence, in a plant homozygous from a genetic standpoint, such as the normally self-pollinated barley, the two reproductive systems present in the sac differ only in the number of reduced sets of chromosomes. Brink and Cooper (*2*) found that in alfalfa the endosperm nuclei of cross-fertilized seeds increased significantly faster than those of self-fertilized seeds, while in the embryo there was little difference in the rate of growth following self- and cross-pollination. It is suggested that the normally more rapid growth of the endosperm is due to the stimulus derived from an extra chromosome set rather than from one different in character.

Bělehrádek (*1*) stated that Van't Hoff's law may be applicable to a biological process when the temperatures are not too close or too far from the optimum and also that systematic variations of Q10 with temperature are a constant and general feature, the value of the coefficient generally increasing rapidly at low temperatures. This increase is illustrated in the Q10 value for endosperm growth between 5° and 15° C., as shown in table 1.

SUMMARY

The following observations were made on hand-pollinated Manchuria barley (*Hordeum vulgare* var. *pallidum* Ser.) grown at constant temperatures for 6 days in the greenhouse.

The most rapid pollen tube growth occurred at 30° and 35° C. where the male nuclei were present in the egg sac 20 minutes after pollination. At 5°, 140 minutes were required to reach this stage.

In countable stages, nuclear divisions occurred about 3 times as often in the endosperm as in the embryo. It is suggested that an extra set of genes in the fusion nucleus may be a major factor in the rapid growth of the endosperm.

The endosperm nuclei divided 2 or 3 times in 6 days at 5° C., and 8 times in 1 day at 30° and 35°. In the embryo there was no divison in 6 days at 5°, and 7 divisions in 2 days at 30° and 35°.

At 40° and 45° C., no endosperm divisions were found.

Two young but dying or dead embryos were found at 40° C. No embryo divisions were found at 45°.

Indications of injury to the egg and polar nuclei were present after exposure of 1 hour at 40° C.

Blasting attributed to high temperatures may be due as much to ovule injury as to pollen killing.

Optimal growth of both endosperm and embryo lies in the neighborhood of 30° C. (86° F.). Growth is less rapid at 35°., and death occurs at 40°.

Between 10° and 30° C., growth as measured by number of generations in endosperm and embryo conformed to Van't Hoff's rule on the relation of velocity of reaction to temperature.

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MECHANICAL DETERMINATION OF THE JUICINESS OF MEAT[1]

By BERNARD TANNOR, *junior chemist*, NANCY GRISWOLD CLARK, *formerly junior home economics specialist*, and O. G. HANKINS, *senior animal husbandman, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

Flavor, juiciness, and tenderness are the most important of the factors that affect the palatability of meat. Adequate mechanical methods are available for determining tenderness, but the customary means of testing for flavor and juiciness is to submit samples of the meat to a group of judges for their opinion. The judges taste the small portions of meat and individually grade them in accordance with their own standards. Usually a chart is used as a guide to judgment and for recording opinions. The average of the opinions of the persons constituting the group has generally been accepted as the best measure of the factor under consideration.

It has long been recognized that this method has serious limitations. Individuals differ in their standards for evaluating the palatability factors and, in fact, the standard of the individual varies from time to time. Experience shows that few judges are able to discriminate well within narrow limits and that there is often a tendency to avoid extremes and to follow, more or less subconsciously, what seems to be a safe policy, that of giving intermediate grades or scores. Perhaps one of the greatest obstacles to accurate grading of any one factor of palatability is the influence of the other factors. For example, it is believed that a high degree of flavor and tenderness in a sample of meat would influence many judges to assign a higher grade for juiciness than would otherwise be the case. Possibly also the saliva of the judge would influence his decision, for its flow is known to be variable, depending on other factors of palatability, especially flavor, and on the physical condition and mental attitude of the judge. It seems logical to conclude that the diluting action of the saliva would influence the opinion of the judge with respect to juiciness.

In recent years there has been an increasing realization of the need for mechanical or chemical methods for measuring the palatability factors of meat, to replace, or at least to supplement, tests based on the opinions of judges. The principal developments in technique for measuring palatability factors have been those relating to tenderness. The juiciness factor is more complex, and efforts to develop a labora-

[1] Received for publication August 17, 1942. This study was conducted as part of the National Project Cooperative Meat Investigations. The early stages of the research leading to the development of this method were conducted as part of a cooperative project with the Bureau of Home Economics of the U. S. Department of Agriculture.

[2] The authors acknowledge their indebtedness to Edna V. Steely, of the Bureau, for statistical assistance.

tory technique for measuring this characteristic have not progressed so rapidly.

REVIEW OF LITERATURE

Several methods of determining the juice content of meat have been proposed. All involve the principle of pressure but differ in the design of the apparatus and in the mode of sampling. The early work relating to expressible juice was done on raw meat exclusively and involved no quantitative considerations with respect to comparisons of different samples. In 1905, Grindley and Emmett (*8*) [3] reported that they had obtained about 34 percent of the juice from raw beef by grinding and then pressing the meat in a compound screw press. In 1908, Bigelow and Cook (*1*) cut small pieces of beef round and chuck and pressed them through cotton bags in a glycerin cylinder press. In 1912, Botazzi, as reported by Child and Fogarty (*4*), obtained 40 and 63 percent of fluid from smooth and striated muscle, respectively, by first grinding the raw ox muscle with diatomaceous powder and then applying pressure of 350 atmospheres.

As far as the authors are aware, the first mechanical method developed for the study of juiciness of cooked meat was reported in 1934 by Child and Baldelli (*2*) of the Minnesota Agricultural Experiment Station. By means of an apparatus, called a pressometer, they subjected 2- to 3-gm. samples of meat, wrapped in unsized filter cloth, to a pressure of 250 pounds. The loss in weight of the samples they regarded as indicative of the quantity of juice originally present. The juice absorbed in the cloth was used for chemical analysis. Employing this method over a period of time, Child and her coworkers conducted studies on the effects of temperature of cooking (*2*), freezing (*6*, *10*), type of beef roast (standing rib as compared with rolled rib roast) (*3*), and location of sample (*5*), on the expressible juice content of the meat. Modification of the method involving the use of different pressures in the pressometer was also studied (*2*).

In addition to the pressometer employed at the Minnesota station, a well-known make of hydraulic laboratory press has been used in several institutions. With this apparatus Hall and others at the Kansas Agricultural Experiment Station obtained juice from raw as well as cooked meat by stratifying alternate layers of ground meat and filter paper in the test cylinder.[4] From 18 to 25 percent of juice was expressed from cooked beef, but no significant correlation was found between the proportions of juice pressed from the raw and cooked lean tissues or between either of them and the judgments of a palatability committee as to the juiciness of the samples.

Noble and coworkers (*9*) applied 3,800 pounds of pressure per square inch to pieces of cooked longissimus dorsi and to a composite of certain muscles of the round of beef, which had previously been used for penetrometer readings, and obtained 15 percent of juice. With their method they studied not only different cuts but also samples cooked to different internal temperatures.

Utilizing the hydraulic laboratory press just referred to, one of the present authors [5] conducted a large number of experiments (*11*,

[3] Italic numbers in parentheses refer to Literature Cited, p. 412.

[4] Mimeographed report of the Kansas Agricultural Experiment Station to the Conference on Cooperative Meat Investigations, representing the United States Department of Agriculture and State agricultural experiment stations, 1934, p. 12.

[5] Nancy Griswold Clark, with the technical assistance of Virginia Weatherby, of the Bureau of Home Economics.

pp. 9–10), some of which are unpublished, to determine the optimum conditions for obtaining maximum expressible juice with minimum loss in the apparatus. Different materials, including asbestos wool, felt mats, filter paper, shot, sponge rubber and flat rubber mats, airplane cloth, and bolting silk, were placed with the meat in the cylinder as an aid in separating the juice from the muscle tissue. Different periods of time of applied pressure and different temperatures of both meat and apparatus were also studied. The procedure finally developed consisted in grinding hot cooked meat, wrapping a 50-gm. aliquot in an 18-inch square of bolting silk, placing the sample between two thin rubber pads in the cylinder, and pressing at a total of 10,000 pounds for 5 minutes. Some study was also made of the relation of the juice expressed in this way to the scores of a palatability committee, but a significant correlation was not found.

From this review of the literature it is apparent that the juice of meat has been obtained by pressure and has been analyzed physically and chemically, but there is no indication that any of the methods replaces the judging of samples for juiciness by a palatability committee. It is the purpose of this paper to present a mechanical method for determining the juice content, or juiciness, of meat, that is believed to possess certain advantages over the technique now available for measuring this factor.

It was the opinion of the authors that in the mechanical method the technique of testing and the conditions under which it takes place should not only yield consistent results but should also be easy to duplicate. In view of the fact that the investigator may wish to study the closely related factor of character of juice, all the extracted juice should be so collected as to be available for examination. The method should measure the same characteristics as are measured by human judges, but the results should be more dependable. The proposed method, which was developed by the authors at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., is described in this paper.

DESCRIPTION OF PROPOSED METHOD

APPARATUS

Figure 1 shows the hydraulic laboratory press used in the proposed method for determining the juiciness of meat. This instrument is of standard make, equipped with electrically heated plates, and capable of producing up to 20,000 pounds of pressure. The steel test cylinder is 2¼ inches in internal diameter and 3 inches in depth. A cross section of the cylinder showing the working mechanism is given in figure 2. In the meat laboratory of the Bureau of Animal Industry a Babcock milk-testing bottle is used as the receiver. By the use of this bottle the estimation, if desired, of the fat content of the juice by a modified Babcock technique is facilitated.

The following modifications of the test cylinder, as purchased, were found to be necessary: (1) Filling with solder the two grooves in the bottom of the cylinder; (2) fitting the base piece with a removable steel tube (figs. 1 and 2) 7½ inches long and ⅛ inch in outside diameter; and (3) adding a support for the base, this support being a steel disk 2½ inches in diameter and 1 inch thick. Other necessary apparatus are a disk of packing rubber 2$\frac{5}{16}$ inches in diameter and ⅛ inch thick and a disk of surgeon's gauze 2½ inches in diameter.

SAMPLING

After the roast is removed from the oven, it is allowed to remain at room temperature for 10 to 15 minutes. Samples are then taken as follows:

In beef, the muscle to be tested is dissected from the rest of the meat and cut perpendicular to the fibers midway between the ends. On each half of the muscle another cut is made parallel to and 1 inch from the cut surface. From each of the two resulting slices a cylindrical section of meat is taken perpendicular to, and from the center

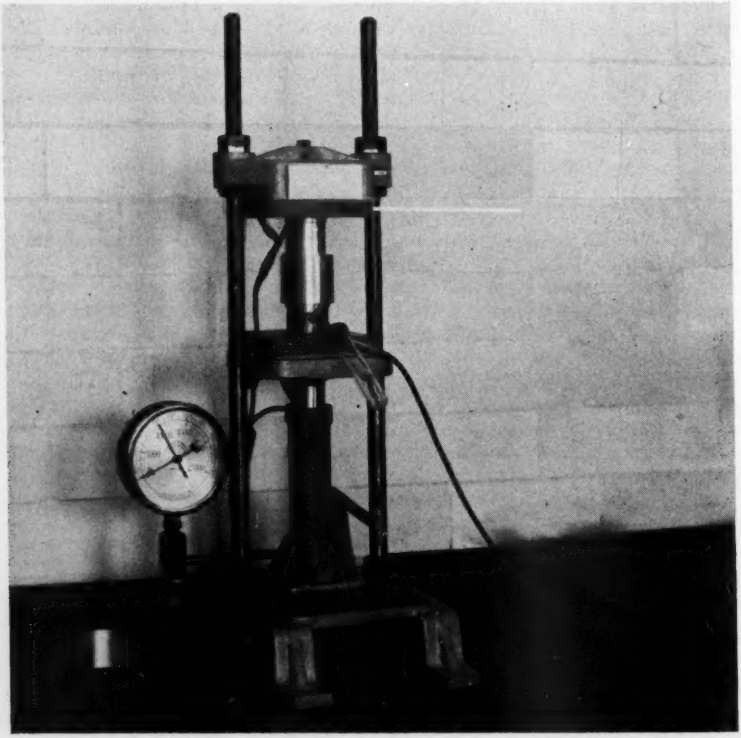

FIGURE 1.—Hydraulic press used in the determination of the expressible juice content of meat.

of, the cut surface with a metal borer having an inside diameter of 1¾ inches. These two cylinders of meat are the samples, in duplicate. Each weighs about 36 gm. Both may be pressed, or one may be pressed and the other sliced for judging by a committee for comparative purposes.

For pork, the procedure is the same as for beef, but owing to the smaller size of the muscles the inside diameter of the borer is 1 inch and the length of the sample is 2 inches. The weight is approximately 23 gm.

In leg of lamb, a cut is made through the popliteal gland as in the standard method of slicing for judging. Another cut is made parallel to this and three-fourth inch toward the pelvic bone, and the slice is then removed from the leg. With the 1-inch borer three pieces are taken from the semimembranosus muscle at positions of uniform distribution in this slice and used as one sample. This sample weighs about 23 gm. The part of the semimembranosus muscle remaining attached to the pelvic bone may be used for committee judging. In the rib and loin of lamb, owing to the small diameter of the muscle, the borer cannot be used. The entire muscle is dissected, trimmed of fat and connective tissue, cut through the center, and a slice 1 inch

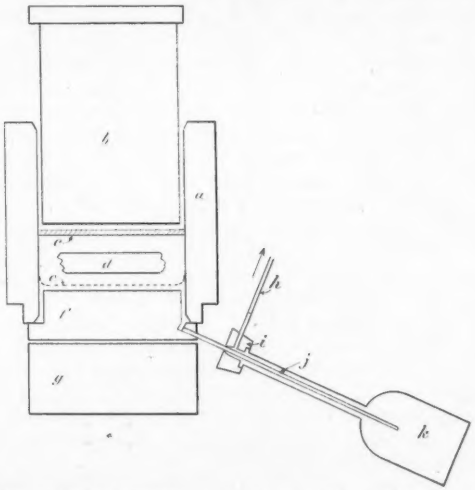

FIGURE 2.—Test-cylinder apparatus in cross section, showing *a*, cylinder; *b*, plunger; *c*, rubber disk; *d*, meat sample; *e*, gauze disk; *f*, base; *g*, support; *h*, suction tube; *i*, rubber stopper; *j*, collecting tube; *k*, receiver.

thick from each half is taken, one being used for pressing and the other for committee judging.

All samples, as soon as taken, are placed in aluminum moisture dishes and kept in an electric oven at 45° to 50° C. until ready for the press.

PRESSING

The hot plates and test-cylinder apparatus are heated to a temperature of 50° C. for the test. The cylinder is placed on the base and the gauze disk inserted (fig. 2). The sample of meat is placed in the cylinder, the rubber disk tucked in over it, and the plunger inserted. The receiver is connected to the tube and the assembled outfit placed in the press on top of the metal disk in order to clear the tube (fig. 2). The connection of the receiver to the source of suction is by means of a tube. The suction is turned on just enough to prevent the leakage of juice around the base of the cylinder. Pressure is applied very slowly.

Juice flows profusely into the receiver until the gage registers about 2,500 pounds. From this point on the pressure is so increased that the juice trickles out at the rate of 1 drop every 2 or 3 seconds, until a pressure of 9,800 pounds is reached. The suction is increased slightly and the pressure maintained at 9,800 pounds (approximately 2,500 pounds per square inch of cylinder area) for 5 minutes. Then the pressure is released and the press cake removed from the cylinder. The loss in weight of the sample is taken as the weight of expressed juice.

ACCURACY OF METHOD

In the development of this method, use was made of numerous pairs of samples that were believed to be as nearly alike in juice content as could be obtained. These samples were taken, for example, from the same muscle or the corresponding muscles from the left and right sides of the carcass. As evidence of the small error involved in the method, part of which undoubtedly is due to sampling, there are given in tables 1 and 2 data from paired four-vertebrae roasted pork-loin samples from 18 animals. In table 1, differences between paired adjacent samples ranged from 0 to 9.9 percent and averaged 2.4 percent. In the paired samples from the left and right sides of carcasses (table 2) the differences ranged from 0.1 to 9.6 percent and averaged 2.9 percent. According to the *t* test of Fisher (7), neither the difference between means in table 1 nor that in table 2 is significant.

In table 1, only two of the differences between adjacent samples exceed 5 percent, whereas in table 2, involving samples from left- and right-side cuts, there are eight such differences. This situation seems logical since it is more probable that adjacent samples of meat from the same muscle will be in the same physical condition than samples of corresponding muscles from the left and right sides of the carcass. Any variation in heat penetration, for example, would be a less disturbing influence in the former.

TABLE 1.—*Expressible juice content of adjacent roasted pork-loin samples tested by the proposed method*

Sample pair No.	Juice content of—		Difference in juice content between samples	Sample pair No.	Juice content of—		Difference in juice content between samples
	Sample *a*	Sample *b*			Sample *a*	Sample *b*	
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	30.9	28.5	2.4	19	40.9	34.5	6.4
2	29.6	26.3	3.3	20	42.6	32.7	9.9
3	30.2	28.7	1.5	21	34.5	38.0	3.5
4	33.6	32.0	1.6	22	41.1	40.7	.4
5	37.5	37.5	0	23	37.8	35.7	2.1
6	36.8	33.8	3.0	24	38.1	35.9	2.2
7	32.0	32.8	.8	25	37.4	32.6	4.8
8	32.4	31.3	1.1	26	43.0	38.4	4.6
9	30.0	33.6	3.6	27	32.7	34.7	2.0
10	37.0	36.9	.1	28	33.6	28.8	4.8
11	40.0	37.9	2.1	29	35.2	34.8	.4
12	38.2	39.2	1.0	30	33.2	34.4	1.2
13	35.7	33.8	1.9	31	37.3	34.6	2.7
14	34.2	33.7	.5	32	40.9	41.3	.4
15	39.2	39.6	.4	33	40.0	37.2	2.8
16	39.3	38.9	.4	34	35.4	33.8	1.6
17	39.6	37.7	1.9	35	29.7	31.7	2.0
18	33.0	28.1	4.9	36	28.2	33.0	4.8
				Mean	35.9	34.5	2.4

TABLE 2.—*Expressible juice content of samples from corresponding left and right roasted pork loins listed in table 1*

Sample pair No.	Juice content of—		Difference in juice content between samples	Sample pair No.	Juice content of—		Difference in juice content between samples
	Left loin	Right loin			Left loin	Right loin	
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	30.9	29.6	1.3	19	40.9	42.6	1.7
2	28.5	26.3	2.2	20	34.5	32.7	1.8
3	30.2	33.6	3.4	21	34.5	41.1	6.6
4	28.7	32.0	3.3	22	38.0	40.7	2.7
5	37.5	36.8	.7	23	37.8	38.1	.3
6	37.5	33.8	3.7	24	35.7	35.9	.2
7	32.0	32.4	.4	25	37.4	43.0	5.6
8	32.8	31.3	1.5	26	32.6	38.4	5.8
9	30.0	37.0	7.0	27	32.7	33.6	.9
10	33.6	36.9	3.3	28	34.7	28.8	5.9
11	40.0	38.2	1.8	29	35.2	33.2	2.0
12	37.9	39.2	1.3	30	34.8	34.4	.4
13	35.7	34.2	1.5	31	37.3	40.9	3.6
14	33.8	33.7	.1	32	34.6	41.3	6.7
15	39.2	39.3	.1	33	40.0	35.4	4.6
16	39.6	38.9	.7	34	37.2	33.8	3.4
17	39.6	33.0	6.6	35	29.7	28.2	1.5
18	37.7	28.1	9.6	36	31.7	33.0	1.3
				Mean	35.1	35.3	2.9

One of the possible sources of error in comparing left and right samples after heating is the layer of external fat on the cut of meat. It is not unusual, after the back fat has been removed in the abattoir, to obtain a pair of pork-loin cuts one of which has a considerable and even layer of fat whereas the other has either a thinner fat layer or an uneven one, muscle being exposed in some places. Furthermore, it is not always possible to obtain cuts that are of exactly the same size, shape, and weight.

EVALUATION OF METHOD

To represent an actual advance in technique, a mechanical method for measuring a palatability factor of meat must enable the investigator to obtain more dependable, significant data than can be obtained from the determinations of judges. However, it is necessary to evaluate the mechanical method by comparison with committee judging.

For such a comparison, 39 fresh beef-round samples, 30 fresh pork-loin samples, and 27 freezer-stored pork-loin samples were used. The cut of round of beef, 4 inches thick, was heated in an electric oven at 125° C. to an internal temperature of 58°. From each cut, three samples were taken, one from the semitendinosus and two from the semimembranosus muscle. Each of these samples was divided into two parts. One-half furnished the slices that were judged by a committee of four to six persons, and the other was used to determine the expressible juice content by the mechanical method. The pork loins were heated at 150° C. to an internal temperature of 84°. In a manner similar to that employed with beef, a section of the longissimus dorsi muscle was removed and one-half was used for the judges, while the other half furnished a sample for the determination of expressible juice content.

Samples were scored by the judging committee as follows: 5, juicy; 4, moderately juicy; 3, slightly juicy; 2, slightly dry; 1, dry. For each sample the scores of the several judges were averaged to give a

committee score. For the beef samples the committee scores ranged from 2.3 to 4.3 and the expressible juice content from 29.3 to 55.3 percent. For the fresh-pork samples the corresponding ranges were 1.0 to 3.8 and 35.9 to 50.0, and for freezer-stored pork they were 1.5 to 3.5 and 34.9 to 50.2.

The coefficients of correlation between committee scores and the percentages of expressible juice for the three groups of samples were as follows: Fresh beef, $+0.63 \pm 0.07$; fresh pork, $+0.60 \pm 0.08$; freezer-stored pork, $+0.58 \pm 0.09$. The foregoing coefficients of correlation obviously are not high. However, they were regarded by the authors as of sufficient magnitude to be encouraging.

In considering the coefficients, attention was drawn to the relatively narrow ranges of committee score for juiciness and percentage of expressible juice. It was believed that with wider ranges of these factors a better measure would be obtained of the relation between them. Therefore an experiment was set up with a view to effecting wide differences in juiciness. One group of pork-loin cuts was heated to an internal temperature of 60° C., another to a temperature of 85°, and a third to 100°, at an electric-oven temperature of 150° in all instances. In the first group, containing 32 samples, the ranges in committee score and percentage of expressible juice were from 2.5 to 4.3 and 47.3 to 57.7, respectively. For the 18 samples in the second group the respective values were 1.5 to 3.5 and 35.7 to 48.0, and in the third group, containing 26 samples, the ranges were 1.0 to 2.8 and 24.7 to 42.5. For all 76 samples, the range in committee scores was from 1.0 to 4.3 and in expressible juice content from 24.7 to 57.7 percent. Similar procedures were applied to rib and loin cuts of beef and lamb.

Correlation coefficients were calculated and equations of the form $y=a+bx$ for estimating committee score (y) from percentage of expressible juice (x) were derived. Table 3 shows, for the three types of meat investigated, high coefficients of correlation, with little variation among them, and a wide range in committee score. The lamb meat varied as widely as the pork in juiciness, and the scores on beef covered the full range, from 1.0 to 5.0.

TABLE 3.—*Relation between (1) committee score for juiciness and (2) expressible juice content of three kinds of meat*

Kind of meat	Samples	Committee score (y)		Expressible juice (x)		Coefficient of correlation and probable error	Estimating equation	Standard error of estimate
		Range	Mean	Range	Mean			
	Number			*Percent*	*Percent*			
Pork loins	76	1.0–4.3	2.73	24.7–57.7	43.29	0.89 ± 0.01	$y=0.089x-1.129$	0.326
Beef ribs and loins	68	1.0–5.0	3.60	17.7–55.0	41.49	$.92 \pm .01$	$y=.113x-1.070$	.437
Lamb ribs and loins	54	1.0–4.3	2.94	18.5–51.8	38.22	$.85 \pm .02$	$y=.084x-.256$	.398

In this comparison of the percentages of expressible juice and committee scores, it was found that pork, beef, and lamb having the same percentages of expressible juice were judged by the committee to differ considerably in degree of juiciness as perceived by taste (table 4). In most instances, as shown by the table, with a given percentage of expressible juice, beef was rated by the judges as being the most

juicy and pork the least. For instance, pork containing 40 percent of expressible juice was judged to be only slightly more juicy than beef or lamb containing 30 percent of expressible juice. Beef containing 45 percent of juice and lamb containing 50 percent were considered by the judges to be practically of equal juiciness. Thus the kind of meat was found to influence human perceptions of juiciness.

TABLE 4.—*Committee scores, for estimated juiciness, of pork, beef, and lamb corresponding to indicated percentages of expressible juice*

Percent of expressible juice	Committee scores for estimated juiciness of—			Percent of expressible juice	Committee scores for estimated juiciness of—		
	Pork loins	Beef ribs and loins	Lamb ribs and loins		Pork loins	Beef ribs and loins	Lamb ribs and loins
25	1.1	1.7	1.8	40	2.4	3.4	3.1
30	1.5	2.3	2.3	45	2.9	4.0	3.5
35	2.0	2.9	2.7	50	3.3	4.6	3.9

SUMMARY

A mechanical method for determining the juiciness of cooked meat has been developed at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. It involves the use of a specially adapted hydraulic laboratory press for determination of the expressible juice content of samples weighing approximately 25 to 35 gm. Pressure on the sample in the test cylinder of the press, maintained at 50° C., is increased gradually to a total of 9,800 pounds and held there for 5 minutes. The juice is collected in a receiver, which may be a Babcock milk-testing bottle, thus facilitating subsequent determination of the fat content, if desired, by means of a modified Babcock technique. The difference in weights of the sample before and after pressing represents the quantity of expressible juice.

In a study of the accuracy of the new method, it was found that adjacent samples from the same muscle and samples from the corresponding muscles of the two sides of the carcass were not significantly different in expressible juice content. Moreover, the coefficients of correlation between committee scores and the expressible juice content of samples of beef that varied narrowly in juiciness, although not high, were of sufficient magnitude to be encouraging. The statement applies also to expressible juice of both fresh and freezer-stored pork. When wider ranges in scores for juiciness of fresh pork and beef were obtained by heating to widely varying internal temperatures, the relationships between such scores and the percentage of expressible juice were close. Likewise a close relationship was found in fresh lamb.

The results indicate that, for the most part, when samples of meat having the same percentage of expressible juice were judged by the committee, beef was rated as being the most juicy and pork the least.

The proposed method appears to be adaptable for use at least with beef, lamb, and pork when the variation in juiciness is due to animal production factors or to the internal temperatures to which the meat is heated. Possibly investigators will find it useful first as a supplement to and later as a replacement of the technique based on committee scores.

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EFFECTIVENESS AGAINST THE CALIFORNIA RED SCALE OF CUBE RESINS IN LIGHT-MEDIUM AND HEAVY SPRAY OILS[1]

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INTRODUCTION

An important characteristic used in grading mineral oils is their heaviness or volatility as indicated by their distillation range. It is generally agreed that the lighter, or more volatile, spray oils are less injurious to citrus trees than the heavy oils, but that they are less effective against the California red scale (*Aonidiella aurantii* (Mask.)) *5, 7*).[2] Rohrbaugh's finding (*6*) that the lighter oils disappear from citrus foliage more rapidly than the heavy oils indicates that the greater tolerance of citrus trees to the lighter oils is due, in part at least, to their more rapid evaporation from the foliage.

In a previous paper (*3*) it was shown that the effectiveness of heavy spray oils against the California red scale on lemons could be increased by combining nicotine or cube resins with the oil. Such results suggested the possibility of obtaining satisfactory control of the scale by means of the lighter oils combined with toxicants. Since from the standpoint of cost, toxicity, and possible phytocidal effects, cube was considered the more promising toxicant with heavy oil, experiments were designed to compare effectiveness of sprays of a light-medium and a heavy petroleum oil containing cube resins. Tests were made in both laboratory and field.

OIL SPECIFICATIONS

The specifications of the oils and their approximate distillation ranges are given in table 1.

TABLE 1.—*Specifications and distillation ranges of the oils used in the experiments*[1]

Oil	Viscosity, Saybolt, at 100° F.	Specific gravity at 20° C.	Unsulfonatable residue	Iodine No. (Hanus)	Acid value
			Percent		
Heavy	91	0.855	96	4.1	0.1
Light-medium	77	.872	100	2.6	.1

Oil	Proportion distillable at temperature (° F.) of—									
	560	575	600	625	636	650	675	700	725	750
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy					5	15	37	61	79	90
Light-medium	5	15	32	49	56	63	76	85	90	

[1] Specifications of the oils were determined by the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine. Data on the distillation ranges were furnished by the manufacturer.

[1] Received for publication August 4, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 419.

FIELD EXPERIMENTS

The methods of preparation of emulsions, application of sprays in the field, and measurement of oil deposits were similar to those previously described (*3*). The oil concentration was 1.5 percent in all treatments. The cube resins, which contained 22.3 percent of rotenone, were dissolved in an intermediary solvent consisting of 1 part by volume of trichloroethylene and 2 parts of dibutyl phthalate and 10 volumes of this solvent was added to 90 volumes of oil before emulsification. This gave a concentration of 1 gm. of cube resins to 4 liters of spray. Four sprays containing light-medium and heavy oils, with and without cube resins, were applied on October 20 in a lemon grove rather heavily infested with the California red scale. Each treatment was applied to a plot of four trees.

OIL DEPOSITS

Each sample analyzed for oil deposit was made up of 100 leaf disks 1.5 cm. in diameter, 4 disks being taken from each of 25 leaves. Eight or nine samples were collected from each plot. The samples were taken on October 21, 24, and 25. It was found that deposits on the samples taken on the last 2 dates averaged lower than those taken on the first date, the difference being 8 percent for the heavy oil and 30 percent for the light-medium oil. This probably represents the amount of oil lost by volatilization. The values obtained on the later dates were corrected accordingly, and the average oil deposits represent estimates of the oil on the foliage the day after spraying. Deposits of heavy and light-medium oil were 141 and 149 micromilliliters per square centimeter, and deposits of the same oils with cube resins, 147 and 135 micromilliliters per square centimeter. The differences between deposits were not significant.

INSECT MORTALITY

Insect counts were limited to the more resistant females; namely, those in the late gray and older stages. Natural mortality in these stages, excluding those scales judged to have been dead before application of the spray, averaged about 5 percent and has been disregarded.

Scales on Wood

As there was not sufficient encrusted material on the twigs to include this type of infestation in the wood counts, the classes of scale densities examined were limited to light and heavy infestations on green and gray wood (*3*). Results of the scale counts made 5 to 6 weeks after spraying are shown in table 2.

Table 2.—*Mortality of California red scales on lemon wood sprayed with heavy and light-medium oil, with and without cube resins*

Kind of wood	Infestation	Heavy oil		Heavy oil + cube		Light-medim oil		Light-medium oil + cube	
		Total scales	Mortality	Total scales	Mortality	Total scales	Mortality	Total scale	Mortality
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Green	Light	718	88.0	504	99.6	771	78.5	486	99.6
	Heavy	767	77.2	782	95.8	762	69.0	902	97.2
Gray	Light	650	78.5	673	95.4	669	68.5	735	96.5
	Heavy	1,542	58.1	2,240	91.2	1,433	51.6	2,140	92.9
Average			75.5		95.5		66.9		96.6

In the analysis of these results the scale counts from each tree were taken as the sampling unit. No replication of plots seemed necessary, since the insects present at the time of treatment were still there when mortality counts were made, and the principal sources of variation in mortality, such as differences in oil deposits, developmental stages of scales involved, population density, type of host surface, and natural mortality, had been controlled or measured by the technique used.

Although the differences in effect between the heavy and light-medium oils were small, analysis showed that they were highly significant ($P<0.01$). When cube resins were added, there was a large increase in mortality and no difference between the effects of the two combinations.[3] Cube resins were just as effective in a light-medium oil as in a heavy oil in spite of the fact that when used alone the heavy oil was more effective. The effect of the oil-cube treatments upon scales in the heavy infestation on the gray wood was outstanding, as it was in the previous experiments reported (*3*).

Scales on Fruit

Mortality of scales on the fruit was determined about 4 weeks after spraying, by examining from 18 to 22 lemons given each treatment. The results are shown in table 3.

Table 3.—*Mortality of scales on lemon fruits*

Treatment	Total scales	Mortality
	Number	*Percent*
Heavy oil	3,878	88.3
Heavy oil plus cube resins	2,224	95.4
Light-medium oil	3,457	82.5
Light-medium oil plus cube resins	3,079	97.3

To permit statistical analysis of the differences between treatments, population densities were calculated, as described in the previous paper (*3*), and were found to range from 0.1 to 8.9 scales per square centimeter.

A trend toward lower mortality at the higher densities of infestation was evident only in the treatments in which cube was not used. The difference between the treatments with heavy and light-medium oils was not statistically significant.[4] However, the difference was in the same direction as that between the counts of scales on the wood and may mean that the heavy oil was slightly more effective against the scales on the fruit even though the differences cannot be shown to be significant. Both oil-cube sprays were superior to sprays containing the same oil without cube. The difference in mortality between the two oil-cube sprays was not significant.

$$t=\frac{(a_1-a_2)-b_c(\bar{x}_1-\bar{x}_2)}{\sqrt{V(a_1)+V(a_2)+(\bar{x}_1-\bar{x}_2)^2V(b_c)}}$$

where a_1 and a_2 denote the mean percentages dead, x_1 and x_2 the mean densities per square centimeter for treatments with heavy and light-medium oil, respectively, and b_c is a combined regression coefficient for the two treatments (*1*).

[3] Since all the mortalities following treatments with oil plus cube were above 85 percent, the inverse sine transformation was made before computing the variance (*2*). This transformation was not used in the analysis of the treatments with oil alone.

[4] The test of significance was made as follows: Population density-mortality regression lines were fitted to the data by the method of least squares after each percentage had been weighted by the reciprocal of its variance. The difference between the means was then tested for significance by computing t from the formula.

LABORATORY EXPERIMENTS

The cube resin used in the laboratory tests contained 27.4 percent of rotenone. It was dissolved in a mixture of trichloroethylene and dibutyl phthalate, as in the field tests. Emulsions were prepared with a high-speed drink mixer and applied with a precision sprayer (fig. 1)

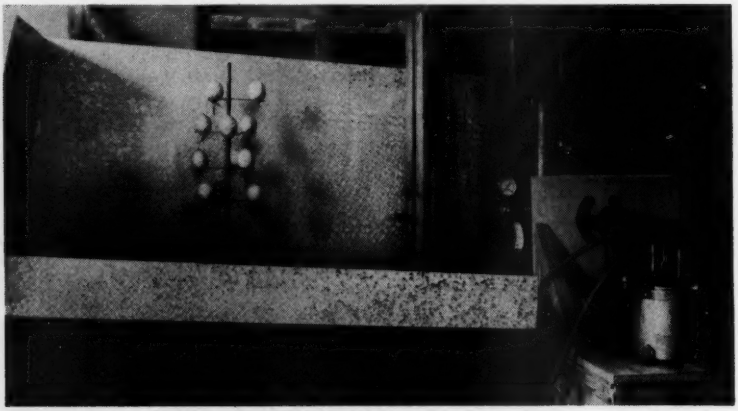

FIGURE 1.—Laboratory equipment used for applying oil sprays on lemons.

similar to that described by Dawsey, Cressman, and Hiley (*4*). Lemons were rested on circular wire supports soldered to a laboratory ring stand, which was mounted on an electrically driven turntable. Each set of lemons was sprayed for 45 seconds at a pressure of 35 pounds per square inch, while the nozzle was moved up and down in a vertical plane and the turntable revolved at the rate of 20 revolutions per minute.

The lemons were infested with scales that had been reared in the laboratory at 25° C. by a modification of a method described by Munger.[5] They were sprayed shortly before the scales began to reproduce, and counts were limited to females in the late gray and older stages, most of them being in the mature stage.

As a result of the method of infestation of these lemons and a rough selection before treatment, it was possible to restrict the range of population densities within rather narrow limits in a given series of spray tests, so that the average mortalities, which are shown in table 5, make a valid basis for comparison of the sprays applied at the same time.

[5] MUNGER, F. A METHOD FOR INFESTING LEMON FRUIT WITH RED SCALE. U. S. Bur. Ent. and Plant Quar. Cir. ET120, 3 pp., illus. 1938. [Processed.]

TABLE 5.—*Effect of adding cube resins to laboratory sprays of light-medium and heavy oils on the mortality of the California red scale* [1]

Spray	Treatments of Jan. 27, oil concentration, 1.5 percent		Treatments of Mar. 15, oil concentration 1.0 percent	
	Total scales	Mortality	Total scales	Mortality
	Number	*Percent*	*Number*	*Percent*
Light-medium oil	1,993	55.4	1,633	57.4±3.01
Heavy oil	1,862	76.3	1,365	61.7±2.84
Light-medium oil plus cube resins	2,000	99.2	1,171	98.0±0.51
Heavy oil plus cube resins	1,781	98.6	1,632	93.4±0.85

[1] Standard errors of the mortalities resulting from lemons sprayed Jan. 27 were not calculated, since it was evident from an inspection of the data that the difference between the first pair of treatments was significant and the difference between the two sprays containing cube was not significant. In the treatments of March 15 the fruits were used as the sampling units in computing the standard errors.

In the first sprays, which were applied January 27 and contained 1.5 percent of oil, the heavy oil was more effective than the light-medium oil when used alone, but when cube resins were added at the rate of 1 gm. to 5 liters of spray there was no difference in their effectiveness. The results of the first laboratory sprays, therefore, were corroborative of the field results, although the differences between treatments were more marked.

In both the field and the first laboratory experiments the mortality from light-medium oil plus cube was slightly higher than that from heavy oil plus cube, but the difference was not statistically significant. The fact that the differences were in the same direction in all cases suggests the possibility that there were slight but real differences in the effectiveness of the two combinations which could not be demonstrated from these two experiments.

Since variability due to uneven coverage and other factors in field sprays might be expected to obscure small differences in efficiency, and the mortality in both oil-cube treatments applied in the laboratory was too high to favor the detection of small differences, the sprays applied March 15 were designed to permit a more satisfactory comparison of the heavy and light-medium oils containing cube resins. Oil concentrations were lowered to 1.0 percent, and the concentration of cube resins was lowered to 1 gm. to 6 liters. In these tests the difference in mortality between the heavy and light-medium oils used without toxicants was less than in the earlier tests, but when cube was added the difference in favor of the light-medium oil was highly significant. The laboratory tests of Marcy 15 were accompanied by measurements of oil deposits on bottles coated with beeswax. No large differences in the oil deposits were found.

DISCUSSION

There are several possible reasons for the greater effectiveness of cube resins in the lighter oil. More of the light-medium oil may have gotten beneath the scale covering, in which case the dosage of cube per scale would have been higher than in the tests with the heavy oil. Furthermore, the light-medium oil may have reached the insect more quickly than the heavy oils. Since cube gradually loses toxicity when exposed to light and air on the plant, a quicker contact with the insect

would be expected to make for greater effectiveness. The possibility of a greater solubility of the toxic principles of the resins in the particular light-medium oil used cannot be entirely ignored. There was no evidence that differences in the oil deposits were a factor in these tests.

In these experiments where efficiency was measured by the kill of the older female scales, one important effect of oil sprays has not been considered, viz, the residual effect of the oil film in inhibiting the settling of young produced by the survivors. It seems probable that the heavier oils, which leave a more persistent residue, would be superior to the lighter oils in this respect. Consequently, the total effectiveness of the light-medium oils and cube resins may not be so great as that of the heavy oils and cube resins, even though a greater increment in effect on the adult scales results from addition of the resins to the lighter oils.

SUMMARY

The relative effectiveness against the California red scale (*Aonidiella aurantii* (Mask.)) of sprays of cube resins in heavy and light-medium petroleum oils has been studied. The cube resins were dissolved in an intermediary solvent consisting of 1 volume of trichloroethylene and 2 volumes of dibutyl phthalate. Insecticidal efficiency was determined on the basis of the mortality of females in the gray adult and older stages. Counts were made on fruit and wood in the field tests, on fruit only in laboratory tests.

In the field tests all sprays contained 1.5 percent of oil. Cube resins were used at the rate of 1 gm. of the resins, containing 22.3 percent of rotenone, to 4 liters of diluted spray. A heavy oil without cube gave a more effective spray than a light-medium oil alone, but a light-medium oil with cube was just as effective as a heavy oil with cube. Mortality on the heavily infested older wood was 51.6 percent with light-medium and 58.1 percent with heavy oil. It was increased to 92.9 and 91.2 percent by the addition of cube resins.

The first laboratory sprays contained 1.5 percent of oil and cube resins, with a rotenone content of 27.4 percent, at the rate of 1 gm. to 5 liters. Mortality of 55.4 and 76.3 percent from a light-medium and a heavy oil was increased to 99.2 and 98.6 percent by the addition of cube. In a second series of laboratory sprays containing 1.0 percent of oil and 1 gm. of cube resins to 6 liters of spray, the differences between the effects of the light-medium and heavy oils without toxicants were less than in the preceding tests, but light-medium oil plus cube was more effective than heavy oil plus cube, mortality being 98.0 and 93.4 percent, respectively.

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